Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: Grazia Pastorelli, Raffaella Rossi, Susanna Cannata & Carlo Corino (2009) Total antiradical activity in male castrated piglets blood: reference values, Italian Journal of Animal Science, 8:sup2, 640-642

To link to this article: http://dx.doi.org/10.4081/ijas.2009.s2.640

Total antiradical activity in male castrated piglets blood: reference values

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ABSTRACT – Blood samples from 146 male castrated piglets in the range of 10-47kg body weight were collected from the same farm and analysed for total antiradical activity in order to determine reference intervals. Data were tested for normality and then submitted to reference limit evaluation. The reference values found in piglets, expressed as half-hemolysis time (59.34 – 93.60 and 43.94 – 66.90 minutes for blood and red blood cell, respectively), are lower than those found in humans; further studies are needed to extend reference values study to female and to animals of different weight classes and different genetic type.

*Key word***:** Reference values, Piglet, KRL test, Oxidative stress.

Introduction - Animal welfare is of concern in order to enhance animal performance and meet the request of consumers for quality and safety of products (Blokhuis *et al.*, 2003). Metabolic, endocrine and immunity parameters are known as welfare indicators, expressing adaptation to the environment. The measure of total antiradical capacity of blood is used in humans as indicator of stress, and can be assessed through KRL test (Prost, 1989, 1992). KRL is a biological test which measures the time needed to hemolyze 50% of the red blood cells exposed to a controlled free radical attack. The principle of the biological test is to submit whole blood to a thermocontrolled free radical aggression in order to mobilize all families of free radical scavengers present in the blood to fight off the oxidant attack (Stocker *et al.*, 2003; Pieri *et al.*, 1996; Girodon *et al.*, 1997). The KRL test have several applications *in vivo*, particularly tested on human studies, or in vitro. In humans KRL is used to study the effectiveness of natural or pharmaceutical treatments or to discover acute processes such as trauma and ischemia or inflammatory disease (Lesgards *et al.*, 2002); moreover it allows to discriminate welfare conditions depending on medium or high stress, or tobacco smoking. According with Brambilla *et al.*, (2002) the response to oxidative stress could be considered as welfare parameter in swine. As a consequence, firstly there is the necessity to establish reference values for KRL test in swine. The aim of the study is to establish blood and red blood cell (RBC) reference values for male castrated piglet in post weaning period.

Material and methods – One hundred and forty six Goland hybrids piglets (all castrated males) in the same range of liveweight (10-30 kg) were selected from the same farm. All piglets were healthy. Blood was drawn by jugular into tubes containing EDTA. All samples were immediately forwarded to the laboratory at a temperature of 6°C and submitted to analysis within 24h of collection. Total antiradical potential for each piglet was evaluated by using a biologic test based on free-radical–induced hemolysis (Laboratoires Spiral, France) (Prost, 1989; Blache and Prost, 1992). Whole Blood and RBC samples diluted to 1/50 was submitted in isotonic saline solution to organic free radicals produced at 37°C under air atmosphere from the thermal decomposition of a 27 mmol/L solution of 2,2´-azobis (2-amidinopropane) dihydrochloride (Spiral, Dijon, France). Hemolysis was recorded using a 96-well microplate reader by measuring the optical density decay at 450 nm. Results was expressed as the time required to reach 50% of maximal hemolysis (half-hemolysis time - HT_{50} - in minutes), which

refers to the whole blood resistance to free-radical attack. Performance of KRL instrument provided by the company, indicate a CV of the repeatability less than 2.5% and of reproducibility less than 4% (Laboratoires Spiral, France). Since haemolysis times vary in a linear way with the trolox concentration, it is possible to convert them in AE (antiradical efficiency) where 1 unit of AE/L of blood or RBC corresponds to the antiradical power of 1 mMol of Trolox /L of reference blood. All data were processed to obtain descriptive statistics and tested for normality by a Kolmogorov-Smirnov test showing Gaussian distribution (P>0.001) and by graphics (Figure 1, 2). Reference values were calculated as mean ±1.96 SD, where SD is the standard deviation from the mean (Farver 1997).

Results and conclusions – Univariate descriptive statistics are summarized in table 1 and reference values reported in table 2. Average HT_{50} expressed as minutes found in male castrated pigs showed the blood values was higher than RBC (table 1) according to the different antioxidant system present in whole blood or in red blood cell analysed; in fact in whole blood, either extracellular or intracellular antioxidant defenses contribute to maintaining blood cell membrane integrity and function until cell lysis (Monaghan *et al.*, 2009).

Table 1. Descriptive statistics of age, body weight (BW) and overall antioxidant capacity, evaluated as half-hemolysis time in minutes (HT_{50}), in castrated male piglets (N=146).

	Mean ± SE	CV[1]%	Median	Mode
Age, days	69 ± 1	29.9	58	58
BW, kg	22 ± 0.9	47.0	20.1	29.00
HT_{50}, min				
- Blood	76.46 ± 0.72	11.41	75.58	76.59
- RBC[2]	55.42 ± 0.48	10.57	54.98	58.06

[1]*Coefficient of variation;* [2]*Red Blood Cell.*

Table 2. Reference values of the overall antioxidant capacity, expressed either as half-hemolysis time in minutes (HT_{50}) either as antiradical efficiency (AE), in castrated male piglets.

	HT_{50}, min	AE eq mMol
Blood, min-max	59. 34 – 93.60	19.82 – 31.28
Red blood cell (RBC), min-max	43.94 – 66.90	14.67 – 22.36

Since the measurement of HT_{50} is very reproducible KRL test has been shown to be representative of the overall defense against free radicals in humans and animal models (Prost, 1989, Girodon *et al.*, 1997, Bourdon 1999). The reference values found in male piglets are lower than those found in humans (84 – 101 and 66 – 75 min respectively for blood and RBC). The lower reference values in piglets may be related to the age, the housing systems and likely, to differences among species. Values of HT_{50} assessed on blood or RBC have different interest for researchers. The analysis on blood allows to measure intracellular and extracellular defense considering the synergic effect of the two actions, whereas RBC analysis concerns especially the intracellular defense status. The two information are complementary. The RBC mean life in pig is 60-85 days, consequently RBC is important for the interpretation of the balance between attack and defence of organism in a medium/long period. On the contrary whole blood parameter gives an indication more immediate of the physiological status. The parameters proposed could be useful especially for the evaluation of welfare in intensive pigs system for studying the influence of housing systems and nutrition. Further

Figure 1 Frequency distribution of half-hemolysis time time (min) in blood.

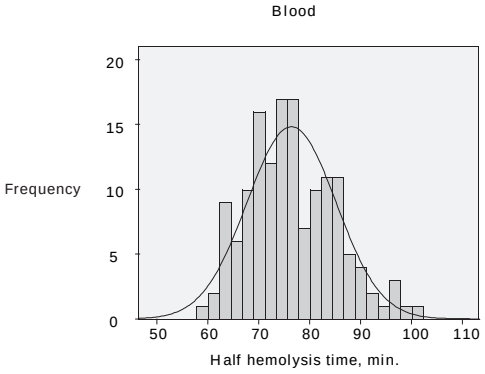
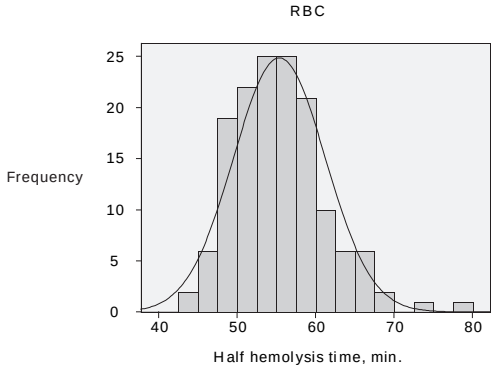


Figure 2 Frequency distribution of half hemolysis (min) in Red Blood Cell (RBC).



studies are needed to extend reference values to female and to animals of different weight classes, genetic types and with different feed rationing plane.

This work has been done as a research project called "Nuove metodologie per la valutazione oggettiva del benessere animale nella specie suina" financed by Regione Lombardia - Agricultural Department, according to the Plan of Research and Development 2007.

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